Effect of Intake of Dried Mackerel on Brain Fatty Acid Composition and Passive Avoidance Performance

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Abstract: This study attempts to determine the effect of feeding mackerel dried by low temperature vacuum method to mice on their learning behavior and fatty acid composition of the nervous system. Thirty-two male mice were fed either a control (palm oil, control group) diet or a diet of 20% dried mackerel (mackerel group) for three months. Learning behavior was assessed using a passive avoidance test, and the tissue fatty acid composition was measured after the behavioral test. The lack of experience with electronic foot shocks resulted in both the control and mackerel groups showing shorter latency during the acquisition trial (Day 1). The avoidance latencies of each group subsequently increased after multiple acquisition trials. The mackerel group showed a significantly longer avoidance latency on day 2 than was the case with the control group ($p < 0.05$). In terms of the fatty acid composition of the serum and nervous tissues such as the brain and retina, the mackerel group showed increased percentages of total n-3 fatty acids, and especially docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids. Meanwhile, the control group showed decreased DHA levels, but increased docosapentaenoic acid (DPAn-6) levels. From the above results, it may be concluded that the intake of mackerel dried by low temperature vacuum improved learning behavior, as assessed by passive avoidance test, with increased DHA in the brain.

Key Words: Brain, mackerel, fatty acid composition, n-3 fatty acid deficiency, passive avoidance test.

INTRODUCTION

The consumption of fish is known to have many health benefits. This is due, in part, to high concentrations of n-3 polyunsaturated fatty acids (PUFAs) including docosaheaenoic (DHA, 22:6n-3) and eicosapentaenoic (EPA, 20:5n-3) acids. Some of the benefits of fish can be obtained by consuming fish oil extracts. Several studies in adults have suggested that fish intake reduces various cardiovascular risk factors through such means as the improvement of lipid profiles, the inhibition of lipid peroxidation, and the reduction of platelet aggregation. In addition, fish is also known to have an anti-arrhythmic effect [1-2]. Siscovick *et al.* [3] have suggested that the effect of fish consumption in terms of the prevention of strokes is mediated by the cell membrane concentrations of the PUFAs. While some studies have shown substantial benefits emanating from fish consumption, others have been for the most part negative. A study conducted by Kromhout *et al.* [4] found a lower instance of deaths related to coronary heart disease amongst those who ate fish. Ascherio *et al.* [2] suggested that while fish consumption was beneficial in conjunction with some endpoints such as fatal and non-fatal coronary heart disease, myocardial infarction, and stroke, it showed no benefits in terms of the prevention of cardiac bypass surgery. Meanwhile, Caicoya [5] reported that increased fish consumption actually entailed a greater risk of stroke.

While the benefits of fish consumption are attributable to higher concentrations of PUFAs, the most beneficial PUFAs are DHA and to a lesser extent EPA [6]. DHA is an essential component for maintaining and improving brain functions in animals that is transported across the placenta and delivered in postnatal milk. Postnatal DHA has been positively associated with visual and language development in breast-fed infants. Helland *et al.* [7] showed that cod liver oil supplements improved children's IQ at 4 years of age. In the present experiment, our purpose was to examine the effect of the intake of dried mackerel, which was enriched with DHA and EPA so as to increase the intake of such n-3 PUFAs, on passive avoidance learning task. The passive avoidance task is considered to be a simple test of leaning and memory in normal mice. A low temperature vacuum dryer was employed to dry the mackerel in a manner which would make it possible to minimize nutritional deterioration. The relationship between learning behavior and brain fatty acid composition was also investigated using adult mice fed with either an n-3 fatty acid deficient diet or mackerel supplements.

MATERIALS AND METHODS

Animals and Diets

Male Crj: CD-1 mice 4 weeks of age were obtained from Charles River Japan Inc. (Atsugi, Kanagawa, Japan). Thirty-two mice were randomly divided into 2 groups of sixteen. These included a control group that was fed a palm oil diet (Control group) and another group that was fed a 20% dried mackerel diet (Mackerel group) (Table **1**) and mice were fed on these diets for three months. The macronutrients, including carbohydrates, protein and lipids, were adjusted in accordance with the amount of mackerel. Mackerel (*Scomber japonicus*) obtained from South Sea, Korea was dried under 40 torr at 40℃ using low temperature vacuum drying system. The dried mackerel contained 33.17% crude

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fat, 59.69% crude protein and 0.43% crude carbohydrates. The main fatty acid composition of the lipids in each diet group is presented in Table **1**. The custom pelleted diets were commercially obtained, and a cold pelleting process was employed to preserve unsaturated fats (Dooyeul Biotec, Seoul, Korea). In the present study, the control diet was n-3 fatty acid deficient diet with deficient in α-linolenic acid and DHA. The diets were stored at –25°C, and fresh supplies were given to the mice once every two days. The diet and water were given *ad libitum*. Body weights were measured once a week. All mice were housed in a standard environment in which the temperature was maintained at 23±1°C, and relative humidity was kept at 65±5% with 12 h periods of light and darkness. This experimental protocol was approved by Korea Maritime University's Animal Care and Use Committee.

Table 1. The Composition of Diet Experimental Diets

Ingredients	Diet Group (g/kg)	
	Control	Mackerel
Corn starch	436	421
Casein	200	81
Sucrose	150	150
Cellulose	50	50
Vitamin mixture[a]	40	40
Mineral mixture[b]	20	20
L-Methionine	2	2
Choline chloride	2	2
Palm oil	100	34
Mackerel	-	200
Selected Fatty acid composition		
Total Saturates	51.9	45.0
Total Monounsaturated	38.3	28.7
Total n-6 fatty acids	9.0	8.2
Total n-3 fatty acids	0.01	15.6
20:5n-3	-	4.5
22:6n-3	-	8.8

Control, 10% palm oil diet; Mackerel, 20% dried mackerel diet.

[a]AIN-93G vitamin mixture contained (in g/kg of mixture): niacin 3; calcium pantothenate HCl 1.6; pyridoxine HCl 0.7; thiamine HCl 0.6; riboflavin 0.6; folic acid 0.2; biotin 0.02; vitamin E acetate (500 IU/g) 15; vitamin B_{12} (0.1%) 2.5; vitamin A palmitate (500,000 IU/g) 0.8; vitamin D_3 (400,000 IU/g) 0.25; vitamin K1/dextrose mixtures (10 mg/g) 7.5; sucrose 967.23.

[b]AIN-93G mineral mixture contained (in g/kg of mixture): calcium carbonate 357; potassium phosphate (monobasic) 196; potassium citrate H_2O 70.78;sodium chloride 74; potassium sulfate 46.6; magnesium oxide 24; ferric citrate, USP 6.06; zinc carbonate 1.65; manganous carbonate 0.63; cupric carbonate 0.3; potassium iodate 0.01; sodium selenate 0.01025; ammonium paramolybdate $4H_2O$ 0.00795; sodium metasilicate $9H_2O$ 1.45; chromium potassium sulfate $12H_2O$ 0.275; lithium chloride 0.0174; boric acid 0.0815; sodium flouride 0.0635; nickel carbonate 0.0318; ammonium vanadate 0.0066; sucrose finely powdered 221.026.

Passive Avoidance Test

When mice were 15 weeks old, passive avoidance test was performed. A step-through passive avoidance apparatus [8] (Gemini Avoidance System, San Diego Instruments Inc., San Diego, USA) was employed to evaluate learning and memory abilities. The box consisted of two compartments of equal size (17 x 12 x 11 cm) separated by a common wall. 20 W bulbs were used to brightly illuminate one of the two compartments from above. The other compartment was not illuminated and had an electrifiable grid floor. A guillotine type door (6 x 6 cm) was located in the center of the common wall to allow the mice to move about freely between the compartments. The door was then closed and the mice were placed in their respective compartments. After a 60 s adaptation period, the compartment lit up and the door opened. Once a mouse entered the dark compartment, the door closed and an electric foot shock of 0.4 mA was delivered for 1 sec. Each mouse underwent one trial, and was assigned a cut-off time of 300 s. The initial latency time required to enter the dark chamber was then recorded. After 24 h, the latency time was measured in the same manner as during the acquisition trial, and was tested on consecutive days.

Measurement of Lipid Composition

After the behavioral experiment, the mice were then decapitated. Tissue samples that included those from the brain and retina were removed and stored at -80℃. The lipids extracted from the tissues were prepared in accordance with the method developed by Folch *et al.* [9]. The lipid extracts were then transmethylated with 14% BF_3-methanol at 100℃ for 60 min using a modified version of the method employed by Morrison and Smith [10] that involved the addition of hexane. Gas liquid chromatography using a 100 m×0.25 mm i.d. ×0.2 μm capillary column (SP-2560, Supelco, Bellefonte, USA) was employed to separate the fatty acid methyl esters, and the latter were detected through flame ionization [11]. The detector and injector temperatures were set to 250℃. The oven temperature program began at 130℃ and increased to 175℃ at 4℃/min, then increased at 1℃/min to 210℃, and finally increased at 30℃/min to 245℃, with a final hold for 15 min. The chromatograms were recorded and the percentage composition of individual peaks was calculated with a VARIAN CP-3380 (Varian Inc., CA, USA). The fatty acid methyl esters were identified through a comparison with the retention times obtained using a standard mixture (37 Component FAME Mix, Supelco, Bellefonte, USA). The percentages of individual and total fatty acids were obtained using an internal standard (22:3n-3 as methyl ester).

Statistics

All results were expressed as means ± the standard error of the mean (SEM), with statistical significance determined by t-test using the SIGMASTAT statistical program package (Jandel Co., Erkrath, Germany).

RESULTS AND DISCUSSION

Fatty Acid Composition of Serum, Brain, and Retina

A difference in the mean percentage of both the DHA and docosapentaenoic acid (DPAn-6, 22:5n-6) fatty acids found in the serum of the two dietary groups was uncovered (Table **2**). The mice fed with a mackerel diet exhibited higher levels of DHA and EPA than the control group. Meanwhile, the mice in the control group exhibited lower levels of DHA and total n-3 fatty acids than did the mackerel diet group, but higher percentages of DPAn-6 and total n-6 fatty acids than the latter ($p < 0.05$). Moreover, the mackerel

group was found to have higher percentages of total saturated fatty acids and lower total monounsaturated fatty acids than was the case with the control group ($p < 0.05$).

Table 2. Effect of Dried Mackerel Feeding on Serum Fatty Acid Composition (wt %) in Mice

Fatty Acids	Dietary Group	
	Control	Mackerel
Total Saturates	31.0 ± 0.53	35.3 ± 1.41*
Total Monounsaturated	31.7 ± 2.11	15.1 ± 1.41*
18:2n-6	11.1 ± 1.02	9.2 ± 0.44
20:3n-6	1.6 ± 0.10	0.3 ± 0.03*
20:4n-6	9.0 ± 0.91	8.1 ± 0.58
22:5n-6	1.1 ± 0.18	0.2 ± 0.02*
Total n-6	23.3 ± 1.50	18.3 ± 0.62*
18:3n-3	0.4 ± 0.05	0.3 ± 0.03
20:5n-3	0.1 ± 0.02	5.0 ± 0.30*
22:6n-3	1.0 ± 0.09	13.7 ± 0.21*
Total n-3	1.6 ± 0.08	19.0 ± 0.27*
Total Fatty Acids (μl/ml serum)	4.7 ± 0.81	3.1 ± 0.45

Data are mean ± SEM values (*n*=5).
Control, 10% palm oil diet; Mackerel, 20% dried mackerel diet. *p <0.05, versus control.

In the brain, the percentage of DHA and total n-3 fatty acids was found to be significantly higher in the mackerel diet group than in the control group (Table **3**). Meanwhile, mice fed the control diet featured lower DHA levels accompanied by increased arachidonic acid (AA, 20:4n-6) and DPAn-6 levels in their total brain lipids. A pattern similar to that of brain fatty acid composition was also uncovered in the case of the retina. While the percentage of AA, DPAn-6 and total n-6 fatty acids, and the ratio of n-6 to n-3 was higher in the control group, the mackerel group exhibited higher percentages of 22:5n-3, DHA and total n-3 fatty acids (Table **4**). No differences were uncovered between the two groups in terms of the percentages of total saturated and monounsaturated fatty acids found in both the brain and retina.

Effect on Passive Avoidance Test

No differences were uncovered between the two experimental groups in terms of body weight and food consumption. Moreover, the acquisition trial (Day 1) revealed no significant differences between the two groups with regards to the latency with which the dark room was entered (control: 22.4±17.4 ; mackerel: 9.0±1.16) (Fig. **1**). Multiple acquisition trials were improved by the supplementation of mackerel diet (Day 2-4). Specially, the mackerel group showed a much more significant increase in avoidance latency on day 2 than the control group ($p < 0.05$). No significant differences in latency were recorded between the two groups after day 2 because both groups had by then, through repeated trials, learned how to avoid the electronic shock.

Table 3. Effect of Dried Mackerel Feeding on Brain Fatty Acid Composition (wt %) in Mice

Fatty Acids	Dietary Group	
	Control	Mackerel
Total Saturates	42.1 ± 2.03	43.7 ± 0.89
Total Monounsaturates	22.8 ± 2.15	23.5 ± 0.84
18:2n-6	0.21 ± 0.01	0.19 ± 0.01
20:3n-6	0.16 ± 0.07	0.28 ± 0.07
20:4n-6	7.44 ± 0.31	5.38 ± 0.12*
22:2n-6	0.41 ± 0.17	0.36 ± 0.22
22:5n-6	6.30 ± 0.25	5.21 ± 0.23*
Total n-6	14.5 ± 0.54	11.4 ± 0.35*
22:5n-3	0.47 ± 0.05	0.82 ± 0.08*
22:6n-3	9.91 ± 0.28	13.1 ± 0.39*
Total n-3	10.4 ± 0.30	13.9 ± 0.44*
Total Fatty Acids (μg/mg brain)	25.6 ± 1.32	25.7 ± 1.72

Data are mean ± SEM values (*n*=5).
Control, 10% palm oil diet; Mackerel, 20% dried mackerel diet. *p <0.05, versus control.

Table 4. Effect of Dried Mackerel Feeding on Retinal Fatty Acid Composition (wt %) in Mice

Fatty Acids	Dietary Group	
	Control	Mackerel
Total Saturates	47.1 ± 0.74	46.7 ± 0.60
Total Monounsaturates	18.9 ± 1.72	14.7 ± 0.97
18:2n-6	0.90 ± 0.14	0.52 ± 0.04*
18:3n-6	0.39 ± 0.14	0.30 ± 0.02
20:4n-6	8.80 ± 0.57	4.20 ± 0.16*
22:5n-6	2.52 ± 0.06	1.20 ± 0.25*
Total n-6	12.6 ± 0.45	6.23 ± 0.24*
22:5n-3	0.47 ± 0.22	0.83 ± 0.04*
22:6n-3	16.8 ± 1.31	22.6 ± 1.17*
Total n-3	17.4 ± 1.49	23.4 ± 1.17*
Total Fatty Acids (μg/mg retina)	8.9 ± 1.99	7.0 ± 0.95

Data are mean ± SEM values (*n*=5).
Control, 10% palm oil diet; Mackerel, 20% dried mackerel diet. *p<0.05, versus control.

From the above results, we found that mice fed with a diet of mackerel were better able to perform passive avoidance tasks than those fed an n-3 fatty acid deficient diet. The improved passive avoidance learning of the mackerel group was associated with higher levels of DHA in brain lipids. These results confirm other studies that have found an increased percentage of DHA in the brain following the provision of dietary supplements containing this fatty acid, and an improvement in mice's performance of the maze-learning

ability test [12, 13]. Garcia-Calatayud *et al.* [14] demonstrated that young rats with a deficint supply of n-3 fatty acid limited to the lactation period presented poor memory retention in passive avoidance test directly correlated to DHA and inversely correlated to brain DPAn-6 levels. Both biochemical and functional consequences of this dietary deficiency were reversed when a supplement of DHA was administered to the deficient animals. This may signify that the increase in DHA and reciprocal decrease in AA and DPAn-6 that occurs within the mice's brain after the latter have been fed DHA are somehow related to improvements in learning ability. Gamoh *et al.* [15] have suggested that the chronic administration of DHA raised DHA percentages in the cerebrum, cerebellum, and hippocampus, and that the ratio of DHA/AA in the cerebral cortex may be considered as an indicator of learning ability. These observations are also supported by other studies in which mice fed an n-3 fatty acid deficient diet exhibited deficiencies in terms of their learning ability [16,17]. Another indicator of the improved learning ability of mice may very well be the fact that the deficiency of n-3 fatty acids induced a decrease in dopaminergic receptors in the synaptic membrane [18].

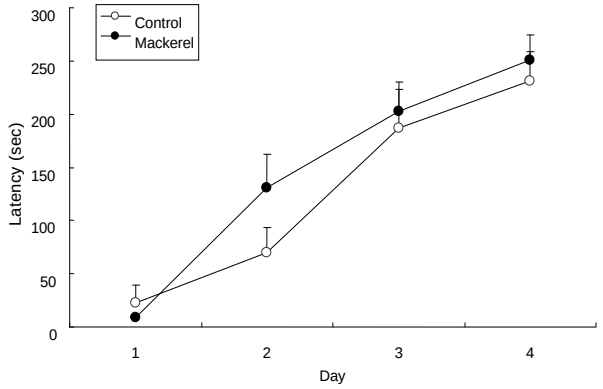

Fig. (1). Effect of dried mackerel feeding on latency in passive avoidance performance. Mice fed the control (palm oil) and mackerel diets for 3 months. Data are means ± SEM values (n=16). *p < 0.05, versus control.

The provision of fish oil or DHA supplements has also been found to improve the elderly's performance on intelligence tests [19] and to reduce aggression in young adults [20]. Studies conducted on humans have shown that the intake of fish may delay the onset Alzheimer's disease, and also that a lower incident of depression was uncovered in patients who regularly consumed fish [21,22]. A recent study using an Alzheimer mouse model found that the intake of DHA inhibits the synthesis of amyloid precursor protein, thereby suggesting that fish oil may also help prevent or delay the progression of Alzheimer's disease [23]. Thus, fish consumption may be beneficial to brain function.

In summary, the passive avoidance test conducted as part of this study found that mice fed mackerel supplements exhibited a 28% increase in brain DHA, as well as a longer response when it comes to staying in the dark room, than those fed an n-3 fatty acid deficient diet. Thus, the improved learning behavior exhibited by the mice fed with mackerel may be associated with an increase in the DHA found in nervous tissues, although our data were preliminary. These results suggest that mackerel dried by low temperature vacuum may represent a useful means of increasing the intake of these n-3 PUFAs.

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